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Hatch Centennial Year Lecture

Plant Hormone Research— A Continuing Challenge





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MARTIN J. BUKOVAC



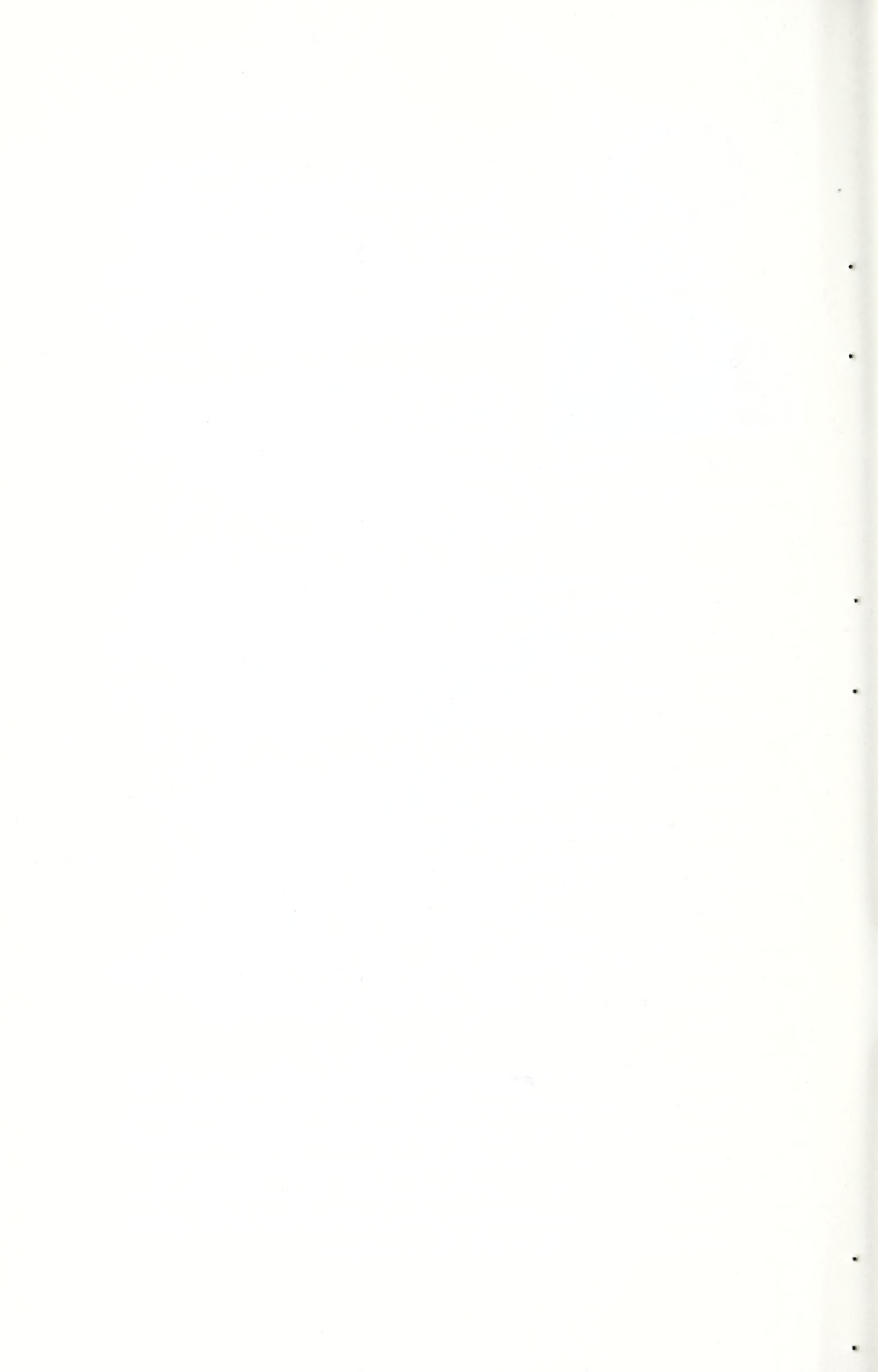
Dr. Martin J. Bukovac is a professor of horticulture at Michigan State University, where he has conducted basic and applied research on plant growth regulators and their uptake by plants. His research has led to patents and new practices in the control of flower and fruit development.

Born in Illinois, Dr. Bukovac grew up in Michigan and attended Michigan State University, earning the B.S. degree with honors in 1951 and the Ph.D. in 1957. He was a National Science Foundation Senior Postdoctoral Fellow at Oxford and Bristol Universities in England during 1965 and 1966.

He was appointed an assistant professor of horticulture at Michigan State University (MSU) in 1957 and became a full professor in 1963. At MSU, he has conducted research on the role of plant hormones on flower formation and on fruit growth and development, as well as on the chemistry, structure, and permeability of the plant cuticle, considered to be the prime barrier to penetration of foliar-applied chemicals.

Dr. Bukovac's professional activities include several major assignments in the United States and abroad. Included are service as National Academy of Sciences exchange lecturer to Council of Academies of Yugoslavia in 1971, visiting scholar at the Virginia Polytechnic Institute in 1973, visiting professor at the University of Osaka Prefecture in 1977 and at the University of Guelph in 1982, distinguished lecturer with the Ministry of Agriculture of the People's Republic of China in 1984, and guest researcher at the University of Bonn in West Germany in 1986. He has also served on several national committees.

Dr. Bukovac was elected to the National Academy of Sciences in 1983 and has been cited for his work on numerous occasions by MSU faculty and by fellow plant scientists nationally. He holds Distinguished Faculty Awards from MSU and from the Michigan Association of Governing Boards. He is a fellow of the American Society for Horticultural Science. In 1974 he served as president of the society, and has been the recipient of its Meadows, Gourley, Blake and Miller Awards.



Plant Hormone Research—A Continuing Challenge

William Henry Hatch Centennial Year Lecture
Presented by

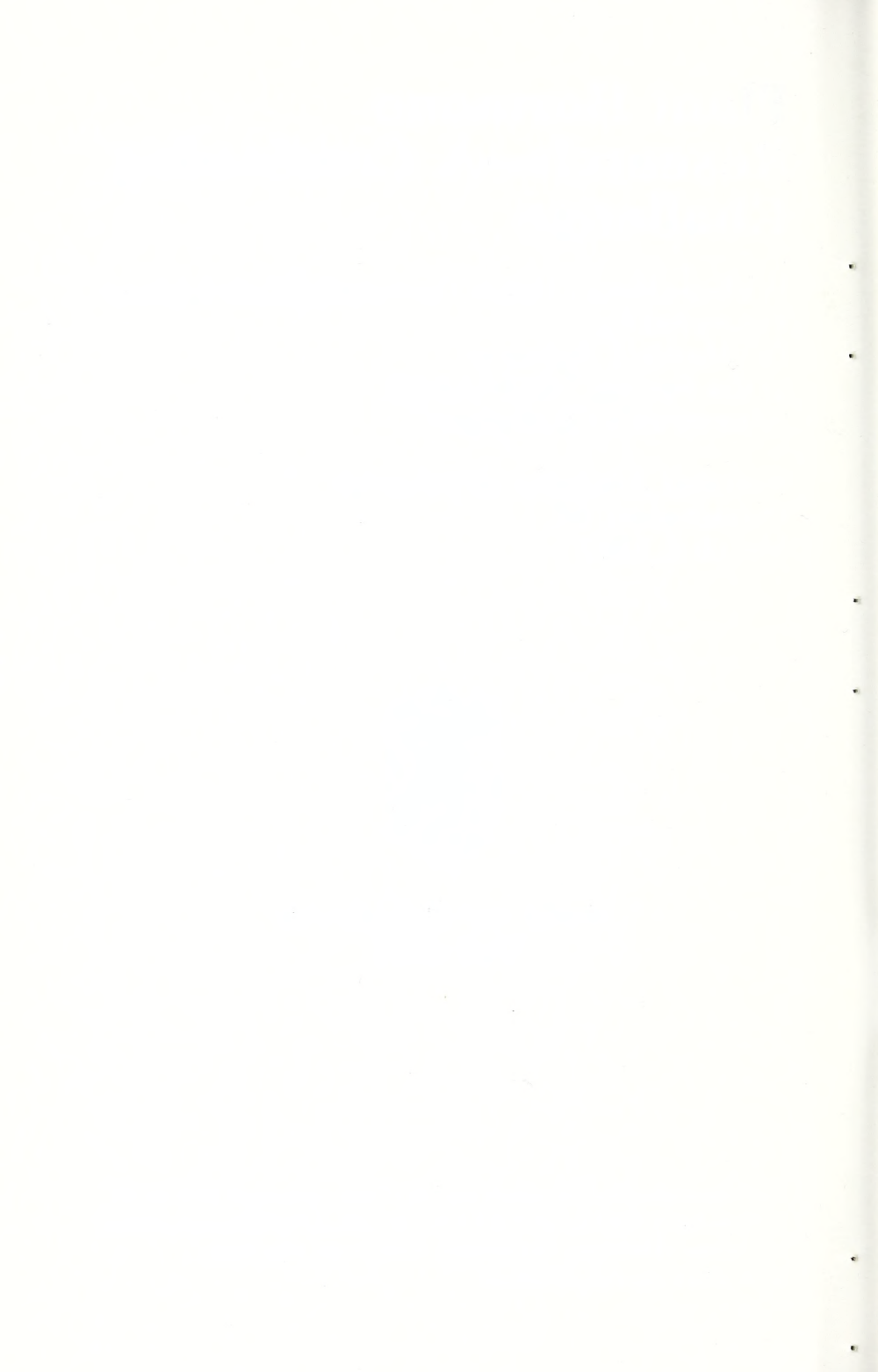
Dr. Martin J. Bukovac
at the Forum, "RESEARCH:
Tomorrow's Challenges"

National Academy of Sciences
Washington, DC
March 2, 1987



**Hatch Act Centennial
1887-1987**

The Cooperative State Research Service, USDA, sponsored this special William Henry Hatch Centennial Year Lecture as part of the official centennial celebration of the Hatch Act of 1887, which created the State agricultural experiment stations.



I am deeply honored to have been selected to participate in this centennial recognition of the enactment of the Hatch Act signed 100 years ago. I am particularly pleased because my entire educational and professional life has been interwoven with the goals and objectives of this far-reaching concept in agricultural research. Celebrations of this kind, especially centennials, provide opportunities to reflect on accomplishments of the past and to dream of the future.

I welcome this opportunity to share with you my thoughts on the exciting area of the regulation of plant growth and development. I have chosen to first reflect on the discovery and impact of these special substances called hormones, and then focus on some challenges we face in the future.

Discovery

The green plant is central to all life on this planet. It carries on the most important chemical reaction on earth — photosynthesis. Solar energy from the sun is captured, converted, and stored in products that provide for our immediate needs or for storage reserves. The photosynthetic reaction has been known, in a general way, for over 200 years. However, the concept that activity in one part of a plant might regulate growth and development in another is far more recent.

Auxin. Charles Darwin, better known for his evolutionary concepts, was among the first to set the stage for studies on regulatory functions in plants. About the time the Hatch legislation was being debated, he and his son Francis demonstrated that covering the tips of grass seedlings prevented their bending towards the light.

Over the next 25 years, studies by several European scientists (Fitting, Boysen-Jensen, Paál, Söding, Kögl) contributed to the concept that some “influence” was produced in the seedling tip, migrated down the coleoptile (the sheath surrounding the first leaf), and there influenced the growth response. Kögl later named this hypothetical substance “auxin.” Less than 60 years ago, Went was able to collect a biologically active substance from tips of oat seedlings by diffusion into gelatin (agar) blocks. When placed on decapitated coleoptiles, these gelatin blocks duplicated the effects of the seedling tips. Biological response was

proportional to the number of tips assayed and the length of time in contact with the blocks. This was a pivotal find, for it not only proved that a growth factor was produced but also provided a means for quantitatively measuring its activity. Ironically, the first identification of the active principle indoleacetic acid, or IAA, was made from urine in 1934 and from yeast and a fungus in 1935. Not until 1946 was IAA identified in higher plants.

Gibberellin. While scientists in the Western World were focusing on auxin as “the growth substance,” fascinating studies were underway in the Orient on a pathogenic fungus, *Gibberella fujikuroi*, that infected rice plants and caused them to grow exceptionally tall. This disease was referred to as the Bakanae, or the foolish seedling disease. In 1928, the Japanese plant pathologist, Kurosawa, showed that extracts of this fungus induced dramatic growth responses in rice plants. About the same time that IAA was identified, Yabuta crystallized the active principle from the fungus and named it gibberellin. Because of war-time secrecy, little was published on the gibberellins. A representative of the Imperial Chemicals Industry brought the information to Britain in the early fifties. Over 70 gibberellins now have been isolated and characterized from both micro-organisms and higher plants.

Ethylene. Before scientists recognized the existence of plant hormones, the simple hydrocarbon gas, ethylene, was known to induce a variety of plant responses — leaf abscission, breaking of dormancy, epinasty, and fruit ripening. Most interesting was the observation that dormancy was broken in potato tubers when stored in a container with ripe apples. The role of ethylene as a plant hormone emerged only after the English scientist, Gane, discovered in 1934 that ripening apples produced ethylene. The idea of a gas being a plant hormone was not popular until the development of the gas chromatograph, which provided a rapid and simple method to detect and measure the low levels found in plant tissue.

Cytokinins. The discovery of cytokinins was an outgrowth of studies by Skoog and associates on growth and differentiation of tissues. Cells of tobacco stem segments would cease to divide after a period of time in culture. However, yeast extract in the presence of auxin (IAA) promoted new and continued cell division. The active principle, 6-furfurylaminopurine, was identified in 1955. Some 9 years later, the first cytokinin in higher plants was isolated from corn kernels by Letham in New Zealand and named zeatin.

Abscisic acid. Inhibitors of growth were frequently encountered during studies on auxins. These interfering substances were first considered a nuisance and given little attention. However, in the 1950's, experiments showing that high levels of inhibitors may be associated with abscission and dormancy stimulated renewed interest. The active inhibitor, abscisic acid, was isolated from young cotton fruits in 1963 and has since been found to occur as a hormone.

Today physiologists believe that the many developmental processes in plants are controlled by representatives of these five classes of hormones, acting individually or, more often, with one another. Numerous other biologically active compounds have been isolated from plants or micro-organisms or synthesized in the laboratory. They are not considered hormones by definition, but many are useful in agriculture and some may play important roles in plant growth and development.

Discovery to Practice

Within a few years after the discovery of auxin, scientists found practical applications of immense importance to agriculture. As other hormones were discovered, they too were rapidly applied, either in commercial practice or as probes to uncover mechanisms of plant growth and development.

These substances offered opportunities for controlling plant developmental processes that could not be readily regulated by any other means. The development and application of this biotechnology, on the one hand, provided tools for a better understanding of plant biology and, on the other hand, modified physiological processes like germination, dormancy, flowering, fruit growth and senescence, which lead to improved food products and increased cropping efficiency.

The following are illustrations of a few applications:

A derivative of auxin, indolebutyric acid, promotes rooting of cuttings. Roots can be induced to develop on excised shoots, which permit rapid cloning of superior plants.

The synthetic auxin 2, 4-D, which selectively kills dicotyledonous weeds, quickly became established as an important herbicide. A new science, followed by a new industry, was born. A new concept in the control of weeds was introduced which alleviated crop stress and markedly increased production efficiency of agronomic and horticultural crops.

Premature abscission of ripening apples can be controlled by a single foliar spray of another synthetic auxin, naphthaleneacetic acid, at 10 to 20 grams per acre. No other cultural practice known can prevent this natural phenomenon.

Gibberellins have or are being developed to:

- stimulate fruit growth in grapes,
- overcome or reverse the expression of viruses,
- alter sex expression, permitting development and maintenance of parental lines for hybrid seed production,
- stimulate production of hydrolytic enzymes, most notably α -amylase in the malting of barley.

Ethylene is now routinely used to:

- ripen bananas and tomatoes,
- induce and synchronize flowering in pineapple, leading to programmed fruit size and harvest,
- promote fruit abscission in tomatoes and cherries to facilitate harvest and preserve the quality of the product,
- stimulate latex flow in rubber trees.

The cytokinins are used to direct the differentiation of tissue in regeneration of plants and in micropropagation through the use of callus or the apical meristem.

Absciscic acid, the most recently discovered hormone, is an important chemical probe for understanding the function of stomata, which regulate gas exchange and water loss from plants. It appears to have a role in plants in:

- sensing environmental stress, particularly that induced by water deficiency,
- unloading photosynthetic assimilates into organs from the transport stream, and
- regulating the levels of mRNAs coding for storage of protein in developing seeds.

Where Do We Stand and Where Do We Go From Here?

Remarkable progress has been made in a very short time in discovering plant hormones, in describing their physiological effects on plants, and in applying them commercially. U.S. agricultural scientists have truly excelled in application of this knowledge in the development of biotechnology. This progress has been based primarily on empirical studies. Our current knowledge is too descriptive. We have made

advances in understanding the morphological and physiological changes induced with hormones, but are woefully lacking in our understanding of the molecular mechanisms controlling hormone-mediated responses.

The most impressive advances have been made in our understanding of the biosynthesis of plant hormones, particularly ethylene and gibberellin. With biochemical and enzymatic approaches, most of the enzymes and substrates have been identified and the pathway elucidated for ethylene biosynthesis. The enzyme responsible for ethylene levels in most plants, ACC synthase, has been isolated and purified, and monoclonal antibodies have been prepared for its detection. Application of enzymology, high-resolution biochemical techniques, genetic mutants, and growth inhibitor probes has led to identification of over 70 gibberellins and their intermediate products.

In contrast to these advances, little progress has been made in elucidating plant hormone action. Perhaps the most impressive gain has been in understanding the role of gibberellin in mobilization of storage reserves in the barley seed. Here, gibberellin produced by the embryo is secreted into the cells of the aleurone layer and there induces the synthesis of hydrolytic enzymes, primarily α -amylase. The enzymes are secreted into the endosperm where they degrade the storage reserves. We now know that the increase in α -amylase is related to increased levels of messenger RNAs in response to gibberellin. Unfortunately, most plant systems are more complex than this model.

The dearth of our understanding of the site of hormone action poses serious limitations. Binding proteins have been described for all of the plant hormones, but no relationship has been conclusively established between binding and response.

There are several reasons for our dilemma; namely—

- Hormones are present in plant tissue at very low levels.
- A single hormone often produces multiple responses, and the same response may be induced by two or more hormones.
- A high degree of interaction exists among the hormones.
- Target tissue changes in sensitivity to a given hormone with development and with changes in the environment in which it grows.
- Higher (economic) plants are complex genetically and morphologically, and our understanding of the basic processes that control plant growth at the molecular level are rudimentary.

- Instrumentation and biological techniques of sufficient specificity and sensitivity are not readily available.
- Research funds are inadequate and a low priority at the national level. In 1985, the Federal Government invested \$2.1 billion in basic human health research and only 5 percent as much in basic plant science research (Lewis, 1986). Prospects for increased funding are not bright. Frank Press, president of the National Academy of Sciences, concluded recently that real growth in funding will not increase significantly in the near future.
- Many agricultural curricula are weak and programs for professional development are limited.

Many opportunities for future research lie in the "new biology." For instance, tremendous progress could be made in our understanding of hormone action if we —

- identified hormone receptors and the sequence of molecular events between hormone binding and response,
- determined the molecular basis of hormone regulation of gene expression, and
- elucidated the regulation of genes encoded for enzymes involved in synthesis, conjugation, and degradation of hormones.

Significant advances in the following areas could have a marked impact on production efficiency and crop quality:

- controlling reproductive processes like flowering, fruit set, and fruit growth, since they represent primary yield-controlling events in many plants,
- improving efficiency of metabolic processes like photosynthesis, partitioning, and translocation,
- modifying plant response to environmental stress, and
- enhancing food and forage quality by altering composition, improving digestibility, and controlling senescence.

Even with significant progress in bioregulation, we will not fully impact on agriculture unless we adequately support the applied research needed to develop efficient practices. I would like to illustrate this point by using spray application technology as an example. Tremendous advances have been made in developing effective pesticides. New chemistries are being researched that are effective at less than 20 grams per acre. Cost of development of a new product is about \$40 million. The primary delivery system is by spraying the foliage. Yet the efficiency of spray deposition on an apple tree with a typical orchard sprayer is about 35 percent. The remaining 65 percent falls to the ground, deposits on

unintended targets, or drifts off, endangering the applicator and contaminating the environment. Even though this practice is central to the application of growth regulators and crop protection compounds in general and may be, if not already, a limiting practice in some cropping systems, *research* on agricultural spray application *is at a low*. Can the next generation of biocompounds be developed into viable products without a dramatic improvement in spray application efficiency?

What Does the Future Hold?

We are most fortunate today. We stand at the threshold of tremendous opportunities. Progress in development of new biological techniques and powerful instrumentation has never been greater, and is providing essential tools for understanding the very basis of growth and development. But these opportunities bring crucial challenges. In the time remaining, I would like to touch on three areas: the experiment station, resources, and the economic and regulatory environment.

Can the agricultural experiment station, as we know it today, effectively cope with the challenges ahead? I believe it can, providing we develop greater flexibility in addressing interdisciplinary approaches to research issues and opportunities. Departmental organization in a traditional university setting does not represent the most effective model for interdisciplinary, mission-oriented research. Multidisciplinary units, established for a fixed time and reviewed periodically, may provide the greater focus needed to address the future problems in agriculture.

Research to solve tomorrow's agricultural problems will require far greater interdisciplinary efforts, more sophisticated instrumentation, highly specialized support staff and facilities, and greater interaction with related sciences and industries than ever before. Careful consideration needs to be given to shared facilities, particularly in making available skilled technical assistants who can interface with agricultural researchers.

Experiment station staff should be complemented with short-term appointments of postdoctoral fellows and visiting scientists. Such appointments would:

- provide flexibility in augmenting priority areas of study,
- introduce new technology and contribute to professional development,
- complement or strengthen existing disciplines, and
- foster multidisciplinary studies by bridging research groups, departments, and colleges.

The restructuring underway in American agriculture will be a factor in the experiment station approach to agricultural research. The Office of Technology Assessment projects that the number of large farm units will increase from 4 to about 14 percent of all farms by the year 2000, and these will represent 90 percent of net farm income. Moderate-sized farms will decrease from about 10 to 6 percent. What impact will such changes have on public support, research priorities and technology transfer? To what extent will large farms establish their own research programs? Will commodity organizations secure matching State and Federal funds to provide grants and contracts to public and private research institutions for their priority projects?

As agriculture restructures, we will face new challenges in technology transfer. The Extension Service, working closely with the experiment stations, has been very effective in delivering technical information, particularly to the small and medium-sized farms. Owners of large farms, however, find the Extension agent too generally trained and often seek information from consultants or directly from the researcher. As the number of large farms increase and as new bioregulators, having special application needs, are developed, effective means of transferring this technology to both large and small growers will be needed.

The availability of traditional resources and models used for their distribution will play a greater role in determining the direction of agricultural research in the future. The trend toward establishing priorities at progressively higher administrative echelons and increased political influence on funding decisions will continue. As this takes place, scientists, graduate students, and skilled technicians will be attracted to areas of greatest opportunity. The agrochemical industry will further impact on experiment station research through jointly sponsored programs in selected areas, perhaps with favorable patent and overhead arrangements. This may cause imbalances, with some scientists having responsibilities in areas of research with limited traditional funding and fewer options for extramural support. The challenge will be to see that this does not happen too often.

Funding for specialized facilities and major instrumentation will not be as readily available to individual program leaders, but greater emphasis will be placed on shared facilities. This trend will accelerate as plant science research becomes more capital intensive.

Funding through competitive grants will increase. Hopefully, scientists will have comparable opportunities regardless of their areas of study. If existing funding priorities cannot be broadened in a reasonable time, two additional areas should be funded: one to support "proposals of merit" regardless of area of study, and the other to provide fellowships for the professional development of agricultural scientists.

We in agriculture need to place a higher priority on development of our human resources. We need to offer more challenging courses, encourage (require?) and sponsor postdoctoral study, increase opportunities for faculty professional development, establish inhouse programs for scientists to "walk in each other's shoes," and establish national agricultural scientists career awards. We must not avoid this challenge! We must succeed in addressing these issues if we are to interact effectively with and capitalize on the findings of related fundamental sciences.

Future development of plant bioregulators will be challenged by a changing agricultural economy and regulatory environment. Only a few years ago, the eighties were being touted as the decade of plant growth regulation, but this did not come to pass! Several major industries increased their research commitment but have since deemphasized or completely eliminated their growth regulator programs. I see three major reasons for this: inadequate basic understanding of hormone action, decreasing profit margins in agriculture, and increasing regulatory requirements for clearance of new compounds. With few exceptions, these factors will continue to throttle new product application.

Because of increased costs and regulatory requirements, industries will increase their focus on the large acreage crops — corn, wheat, soybean, rice and cotton — to recover their research investment before patent expiration. As important as these crops are, valuable food crops such as vitamin-rich fruits and vegetables will not present a significant primary market for product development. Many existing growth regulators, if challenged by the EPA, will probably be abandoned, as was 2, 4, 5-TP (2, 4, 5-trichlorophenoxypropionic acid). Isn't it ironic that because bioregulators are used in low doses and applied infrequently (attributes considered favorable from a health point of view) they are relegated to minor use status, and thus their development is not economically feasible? Some mechanism must be found to develop compounds for markets that cannot support development costs. Modified regulatory criteria, where possible, and extended patent protection should be considered for those willing to make extraordinary investments.

Summary

The future is challenging indeed, but the opportunities have never been greater. The agricultural experiment station system stands central to meeting these challenges and capturing the opportunities. You are a privileged few, for you hold in your hands the key not only to provide an exciting environment in which to elucidate the mechanisms controlling plant growth but also to translate this knowledge into practice for the welfare of humanity. I thank you for this most exciting opportunity.

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